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Cholera in the Perspective of 1966

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TODAY THE WORD CHOLERA evokes more fear among more people of this earth than any other single word. Starvation is the only other word that has the connotation of cholera—death. However, with starvation one is dealing in terms of weeks; with cholera death comes in a matter of hours.

Cholera can be defined as a disease state caused by the *Vibrio cholerae*. The vibrios do not invade the body but are limited to the gut lumen; the disease is typically afebrile. The chief sign is a profuse isotonic diarrhea of rice-water character, with a rate of output of as much as a liter per hour. Skeletal muscle cramps, presumably due to electrolyte loss, and vomiting are early signs in the untreated patient. Cholera is self-limited with a mean time of 4.2 days. The duration of the disease and stool volume can be halved by the oral administration of the tetracyclines.

This fear of cholera is justified because in untreated cases the mortality rate may be as high as 80%. As recently as 1943, the deaths in that 1 year in India alone were more than 450,000. However, as the result of studies by the United States Navy the mortality has been brought to zero in the properly treated patient who has no accompanying organic disease.

For example, in 1964 cholera invaded Saigon. NAMRU-2, the Navy's Medical Research Institute in Taipei, was requested to provide aid. After Vietnam medical personnel were trained at the Chou Quon Hospital, they treated—with no assistance from us—over 7,000 admissions in the subsequent 3 months with a mortality rate of less than 3%. Before arrival of the Navy team the mortality rate was at the 50% level.

For historical reviews, there are the superb bibliographical study of cholera by Pollitzer (1), a recent (1961) book entitled *Cholera* by De (2) from Calcutta, and for those who are more interested in the American scene a book by Rosenberg (3) entitled *The Cholera Years* (1962).

Despite the convincing evidence of John Snow on the Broad Street pump, the only certainty as to the importance of water in the spread of cholera in recent years has been that cholera travels against the current. In the 1947 Egyptian epidemic, the disease first appeared in the Ishmalia area near the Suez Canal. The disease then spread against the current of the canals in the delta of the Nile, reached Cairo, and then raced upstream. In the last few years in the Far East and in western Pacific areas the variant of the organism known as El Tor, named after the Mecca pilgrimage stopping point in the Arabian Peninsula, has spread widely. The usual pattern is for the disease to hit a port area and then travel upstream into the country or island in which it is located. This, of course, argues for spread of the organism by man.

An equally fascinating problem is why the El Tor variant, which was originally found in the Arabian peninsula at the turn of the century, then turned up many years later in the Celebes island of the Indo-

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nesian archipelago but failed to move out of the Celebes for nearly 20 years. The El Tor variant has been responsible for most of the epidemics since 1961. While this variant of the *Vibrio* can be distinguished in the laboratory from classical strains, the disease state produced in man is not discernibly different from that produced by other strains of the *Vibrio*.

Cholera, with its very high mortality rate in the untreated case, has a very low attack rate. The attack rate in the epidemics seen in the western Pacific area in the last few years runs about 1 in 2,000. However, in endemic areas in the Ganges delta the attack rate may be as high as 1 in 200.

Pathogenesis

Let us now consider the genesis of the stool in cholera, or the pathophysiology of the disease. The U. S. Navy has gathered more information on cholera than has any other organization. Starting with the Egyptian epidemic that was studied by NAMRU-3 in Cairo in 1947, studies were continued by NAMRU-2 (which is located in Taipei), in East Pakistan and Bangkok in the 1958 epidemic, in Bangkok in 1959 and 1960, and in the Philippines from 1961 up through the present. Smaller epidemics have also been studied by NAMRU-2 in Korea, Taiwan, Vietnam, Malaysia, and Sarawak. As a result of our 1947 studies in Egypt we pointed out that diarrhea would not occur unless the absorptive capacity of the gastrointestinal tract for water and electrolytes had been exceeded (4). The diarrhea can result from [1] an increased movement of water and electrolytes from blood plasma into the gut lumen, [2] a decrease in the flow of water and electrolytes in the reverse direction from gut lumen to plasma, or [3] a combination of both (Figure 1).

In discussing our 1958 studies in Bangkok we pointed out that cholera might well be due to inhibition of sodium transport from gut lumen to plasma (5). In 1960,

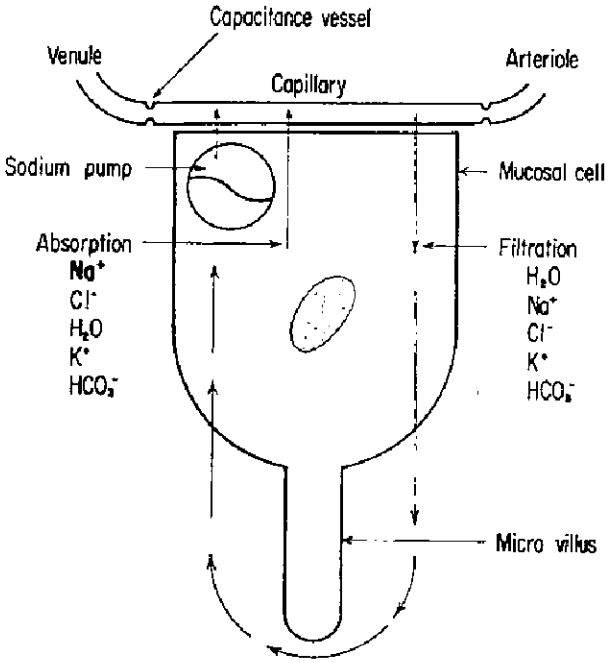

FIGURE 1. Bidirectional flux of water and ions across intestinal mucosal cell. Diarrhea can result from [1] an enhanced plasma to gut lumen flux of protein-free plasma such as would occur with constriction of capacitance vessels; [2] a decrease in gut lumen to plasma flux such as would occur with "poisoning of the sodium pump"; or [3] a combination of 1 and 2. Prepared by the U. S. Navy.

using the short-circuited frog skin preparation of Ussing and Zerhan, Cdr. Huber found that in typical severe classical cholera an inhibitor of sodium transport was usually present in large amounts in the cholera stool (6). This inhibitor was found to be thermolabile; in the same year, independently, Fuhrman and Fuhrman (7) from Stanford reported the presence of a thermostable sodium transport inhibitor in extracts from a laboratory culture of the *Vibrio*.

In addition to the short-circuited frog skin preparation of Ussing and Zerhan, there are several experimental models that have found favor in various laboratories. The suckling rabbit model has been refined by Dutta and Habbu (8); injection of a few vibrios into the jejunum of a rabbit 10 to 12 days of age produces a fatal cholera-like diarrhea—by the age of 30 days there is no diarrhea or fatality. Another

TABLE 1. Electrolyte Concentration in Average Cholera Stool in Adults

Electrolyte	Concentration
	mEq/liter
Na^+	140
K^+	10
Cl^-	110
HCO_3^-	40

preparation found very useful is the in vivo ileal loop of the adult rabbit. In this instance, activity of the *Vibrio* is judged by the accumulation of fluid in the gut lumen, the fluid having the electrolyte composition of the cholera stool.

On the other hand, the results of studies that we conducted on various laboratory models both in vivo and in vitro led us to question the importance of a sodium transport inhibitor in the cholera stool (9). Independently, Burrows, Musteikis, Oza, and Dutta (10) also found that the in vivo rabbit ileal loop did not respond as did the short-circuited frog skin when exposed to various fractions of *Vibrio* cultures.

Let me now tell you some of the things that we do know about the pathophysiology of cholera. Table 1 is the average electrolyte composition of the stool in severe classical cholera. These figures vary by no more than ± 5 mEq/liter. It is evident that we are dealing here with an isotonic dehydration. This information, first reported by O'Shaughnessy (11) in 1831, was also reported by Schmidt (12) in 1850. Sixty years later in Manila, Aron (13) called attention to Schmidt's studies and pointed out the importance of reporting plasma electrolytes, especially in dehydration, in terms of milliequivalents per liter of plasma *water*.

It is not unusual for the cholera patient on admission to have a plasma protein concentration of 14 g/100 ml, and on occasion we have seen values as high as 16 g/100 ml. However, there is no protein "leak" from plasma into the gut (14). Sloughing of the mucosa as seen at postmortem is considered to be the result of shock and not of cholera per se (4).

Figure 2 depicts the constancy of the sodium and potassium concentrations in severe diarrhea and the reciprocal relation that holds in stool outputs below 4 ml/kg

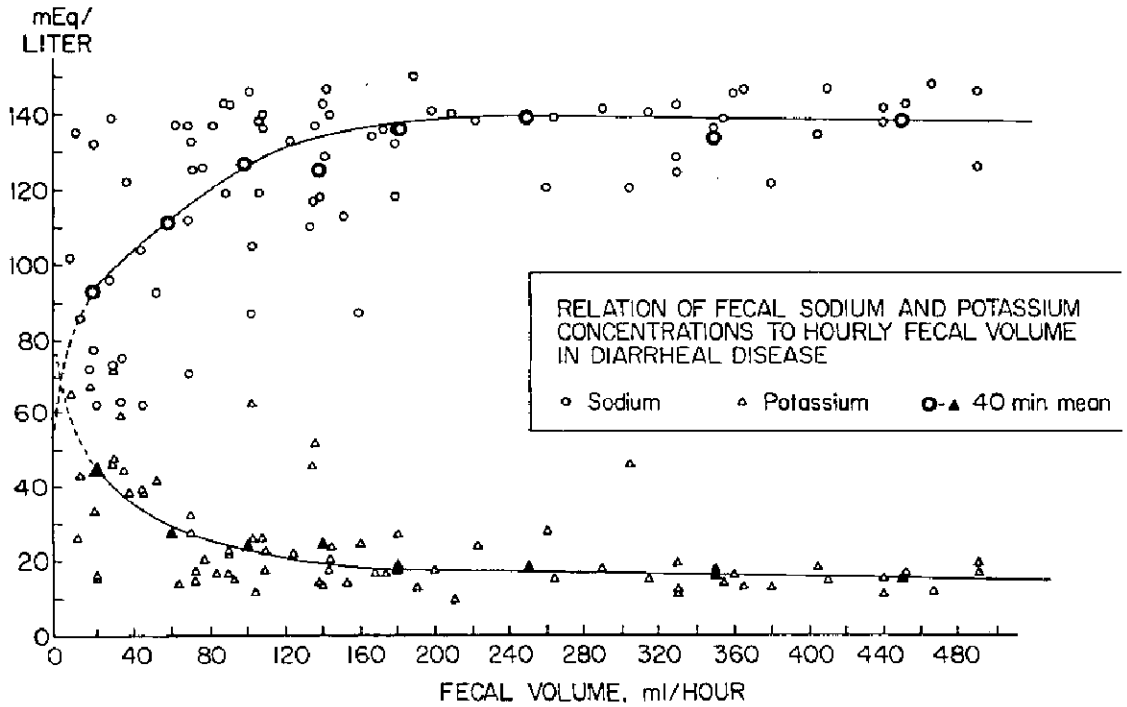

FIGURE 2. Relation of cation concentration to rate of stool output (26). Reproduced by permission of the Pakistan-SEATO Cholera Research Laboratory.

body weight per hr. Similar shaped curves are to be found when the concentration of the chloride and the bicarbonate ions is plotted against stool output.

NAMRU–2 has also conducted studies on the intestinal absorption of cholera patients, studying them both in the acute phase of the disease and again in convalescence (15). In this series of studies the experimental design was basically as follows: The patients entered the study room the first thing in the morning and stool collections were made for 2 to 6 hr while hydration was maintained by an intravenous infusion of a solution that approximated stool output in composition and volume. When a satisfactory base line had been obtained the patient drank an electrolyte solution of known composition for several hours. At the end of this experimental period, a control period of several hours' collections was also made. The first patient to be studied was a patient who was putting out about 700 ml of stool/hr. He was given an electrolyte solution of the same composition as his stool output and he drank this at the rate of 700 ml/hr for a period of 4 hr. Stool output was doubled immediately. There was no evidence that the oral electrolytes or water were absorbed by the patient. In subsequent studies the electrolyte composition of the oral lavage solution was varied, and it was shown that the cholera patient could absorb water when taken by mouth. He also could absorb the bicarbonate and the potassium ions. There was no evidence that the sodium ion could be absorbed in severe cholera, and neither was the chloride ion absorbed. In fact, when a sodium load was given by increasing the sodium concentration to 230 mEq/liter in the oral lavage solution, balance data showed that the patient lost more sodium in the stool in the experimental period than he did in the pre- and postexperimental control periods.

Further, it was found that when D-glucose was added to an oral lavage solution having the electrolyte composition of the cholera stool, the glucose was absorbed, and with its absorption there was also absorption of the sodium and chloride ions. In consequence of this, net stool volume was decreased. This effect is apparent when the oral solution contains 100 mmoles of D-glucose. This oral method of treating cholera is effective in mild cases. However, in severe cases of cholera though the diarrhea can be stopped, D-glucose concentrations of 400 mmoles/liter produce in most instances a syndrome similar to the dumping syndrome with extensive vomiting which precludes continuation of the therapy.

The source of the water and electrolytes in the cholera stool can be readily calculated. A typical male patient weighing 50 kg will have a stool output of 500 ml/hr. This typical patient will have a sodium concentration in plasma water of 150 mEq/liter and a potassium concentration of 4 mEq/liter. Stool concentrations will be 140 mEq/liter for sodium and 12 mEq/liter for potassium; thus 70 mEq of the sodium ion would be in the 500 ml of stool. If this is divided by the concentration of sodium in plasma water, we find that extracellular fluids must have provided 467 ml of protein-free plasma. This is 93% of the volume of the cholera stool. Of the potassium ion there would be a total of 6 mEq in the 500 ml of stool. Four hundred sixty-seven milliliters of extracellular fluid would provide 1.87 mEq of the potassium ion. Subtracting this from the 6 mEq of the potassium ion in the half liter of stool shows that cell water must provide 4.13 mEq of potassium. Dividing this by the 33 ml of fluid that must have come from cells shows that the potassium is leaving the cells at a concentration of 125 mEq/liter of cell water.

A 50-kg male patient with cholera would have about 9 liters of extracellular fluid before onset of the disease. Death is imminent when the subject has lost sufficient fluid to increase his plasma protein con-

centration 2.5 times. In this subject this is equivalent to the loss of about 5.4 liters of body fluid, of which about 5 liters or 10% of his body weight would come from extracellular fluids and 0.4 liters from cell water. According to Wolf (16), in the dehydration observed in shipwrecked sailors, death is not imminent until the body has lost up to 20% of body water. This supports our assumption that in cholera the bulk of the fluid loss is extracellular. The mechanism for the conservation of cell water in cholera is not known. It could be due to the presence of a sodium transport inhibitor in the extracellular fluids, and Cdr. Huber has demonstrated the presence of such an inhibitor in the plasma of a few patients in the acute phase of cholera. We have seen cholera patients put out more than a liter of stool per hour for several hours. Such a patient would be in momentary danger of dying after 5 or 6 hr of diarrhea.

Studies made a little over a year ago by Greenough (17) at the Pakistan–SEATO Cholera Research Laboratory (P–SCRL) are in keeping with the overproduction theory of the genesis of the cholera stool. Greenough passed an intestinal tube with a balloon on the end of it into the area of the ligament of Treitz. He then inflated the balloon and removed by suction the lumen contents above the balloon. Within the hour the diarrhea ceased and he obtained by suction about the amount of fluid the patient had been passing by rectum per hour. This study has been continued by Taylor (18) at P–SCRL with essentially identical results. These studies have suggested that cholera may be due to an increased movement of protein-free plasma from plasma to gut lumen but give no evidence as to whether in cholera there is an inhibition of movement of water and electrolytes from gut lumen to plasma.

In 1964 extensive studies were carried out in Manila and Saigon in an attempt to ascertain if the factor responsible for the diarrhea could be absorbed or chelated. The agents studied were kaolin, activated charcoal, streptomycin, and versene. None of these agents appeared to be of value.

Treatment

The treatment of cholera consists of the volume for volume replacement of the fluids, including the electrolytes contained therein, lost from the body in the course of the disease. Fluids lost through lungs and skin, of course, must be replaced as well. The fluids lost in the stool come directly from the blood; it is most advantageous to give them directly back to the blood. It has been repeatedly shown that with this therapy mortality will be nil in the victim who has no other complicating disease.

Latta (19) in 1831 appears to have been the first person to recognize the importance of intravenous therapy in cholera. However, physicians then, as now, were looking for a cure for the disease, and although they all agreed that after intravenous administration of saline or alkaline saline solution patients could be brought out of shock, this method of therapy did not stop the diarrhea. Since diarrhea continued, the patient would relapse into shock, and this measure—administration of intravenous electrolyte solutions—was considered to be a temporary measure only and not a cure. In consequence it fell into disrepute and was abandoned.

Sir Leonard Rogers (20) was the first to be successful in gaining general acceptance for intravenous therapy in cholera. This was at the end of the nineteenth century. Though Schmidt (12) in 1850 had recognized the loss of bicarbonate ion and the acidosis that resulted, it was not until the rediscovery of this fact by Sellards (21) in 1909 in the Philippines and its adoption by Sir Leonard that rational therapy for cholera had its beginnings. Sir Leonard also recognized the potassium loss in this disease and advocated its inclusion in his

intravenous regimen but withdrew this advocacy a year later after his holidays in the United Kingdom because his physiologist friends said that the rapid administration of an intravenous solution containing potassium might cause cardiac arrest.

Sir Leonard recommended a hypertonic saline solution. However, there were advocates of isotonic saline and of hypotonic saline. Studies by the NAMRU–2 team of the 1958 epidemic of cholera in Bangkok measured these losses in detail for the first time and showed that the loss was an isotonic loss when related to the concentration of electrolytes in plasma water (5). Since Sir Leonard advocated the oral administration of water and other fluids as soon as the patient could tolerate them, it is evident that a hypertonic solution might well have given better results since his patients were also imbibing large amounts of water.

There are two schools of thought on the management of the therapy of cholera today. One is that advocated by the P–SCRL (22). This has shown that the alert, well-trained clinician can estimate fluid requirements purely by following physical signs of the patient without recourse to the laboratory. This approach has also been advocated by the Johns Hopkins University Center Research Team in Calcutta (22).

The other approach is that advocated by the U. S. Navy based on the 1947 studies in Egypt and studies by NAMRU–2 in Southeast Asia and the Far East (23). It has been shown by the Navy scientists that the deficit of fluids at the time the cholera patient is admitted can be estimated with accuracy from measurement of plasma specific gravity by the copper sulfate method (24, 25), and replacement will be approximated if 4 ml/kg of body weight of fluid are given for each increase of plasma specific gravity of 0.001 above the normal value of 1.025 in individuals whose extracellular fluids approximate 18% of body weight; in other words more fluids would have to be given to infants and children. (Whole blood specific gravity, while used by Roy in the 1880's in India, cannot be used with precision because of the varying degree of anemia seen in cholera patients.) In both methods, the patients are placed on canvas cots similar to army cots with a hole for the buttocks so that stool and urine can be collected and measured as frequently as is necessary. Fluids lost are then replaced with intravenous fluids.

The P–SCRL method of therapy requires the presence of an alert, well-trained clinician during the critical time when the patient is being rehydrated and is thus well suited to cholera in the endemic form. Rehydration can be accomplished by the Navy method by unskilled laboratory technicians and nurses without the aid of a clinician. This leaves the physicians, who are usually in short supply, to handle cholera patients who also have complications such as diabetes, heart disease, and kidney disease. The Navy method is thus particularly suited to treatment of cholera in epidemic form in areas where the disease has been absent for years.

It has been widely documented by P–SCRL and NAMRU–2 that the average intravenous fluid requirement in bacteriologically proved cholera is about 20 liters /patient. Patients may have a stool production of as much as 20 liters/24 hr, and it is not unusual for a patient to require one and a half times his body weight in intravenous fluids during the course of his disease (Figure 3).

The use of other medications including cardiac stimulants has no place in the treatment of cholera today, with the exception of certain antibiotics.

Tetracycline has been shown by P–SCRL to shorten the course of the disease and to cut down the fluid requirement. The effectiveness of other antibiotics is now under study by investigators in several countries where cholera is endemic. Their results should be available in the near future. Pa-

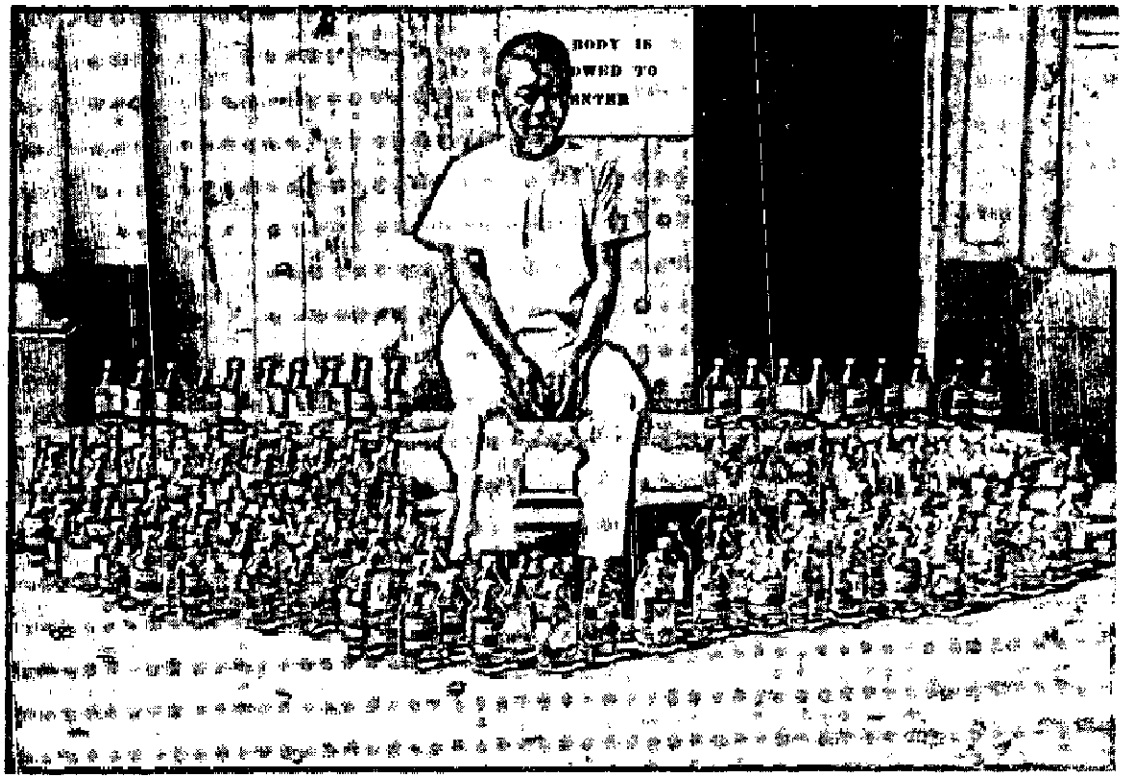

FIGURE 3. Typical cholera patient with empty bottles that had contained the saline solution required for his treatment, seated on the steps of the Cholera Ward at the San Lazero Hospital in Manila, P.I. Official U. S. Navy photograph.

tients with cholera do not die if they are close enough to a good treatment center that they can reach in time. Thus there is an urgent need to explore the possibility that oral administration of D-glucose to patients on their way to the treatment center may prolong their lives so that they are not dead on arrival.

Three years ago the author stated: "There is no evidence that any cholera vaccine is of any value." Today this is not true. Cholera vaccines have been demonstrated to be effective by P–SCRL in East Pakistan. Similarly, the Philippine government working with assistance from Japanese and World Health Organization (WHO) investigators and the Indian government working in collaboration with WHO have demonstrated that cholera vaccines are of value.

The following statement was prepared for this paper by Dr. W. H. Mosely, who is now in charge of vaccine evaluation trials in East Pakistan.

Cholera Vaccine trials were initiated by the Pakistan–SEATO Cholera Research Laboratory in 1963 when a highly antigenic cholera vaccine and a typhoid vaccine control were given randomly to 14,064 persons in 23 rural villages in Matlab Bazar, East Pakistan. Again, in 1964, the same cholera vaccine, a tetanus toxoid control, and a purified Ogawa antigen preparation were given randomly to an additional 25,264 persons in 35 other villages. Surveillance for cholera in these populations has been maintained by a field hospital, and by daily home visits to all vaccinated persons, with collection of rectal swab cultures from all persons with diarrhea. Observations over the past 2½ years have established the effectiveness of a single injection of cholera vaccine in reducing the case rate by 75% in the first 6 months after vaccine administration; however, in the second epidemic, 12 months after immunization, there was a fall in vaccine effectiveness in children, while the vaccine remained effective in adults. Having established the efficacy of a single injection of cholera vaccine, future studies will be directed towards the development of vaccines and immunization schedules which will provide an enhanced and prolonged protection.

Finally, I should like to comment on certain aspects of the economy of cholera. The cost to the cholera victim in East Pakistan today for a course of cholera therapy is equivalent to his wages for a 3-month period. This does not include charges for medical and nursing care.

In the early days of the Vietnam epidemic of 1964, I was requested to estimate the amount of saline that would be required for ensuring adequate supplies. My estimate was based on an attack rate of 1 in 2,000 of the population and a requirement of 20 liters/patient. Saline could not be obtained in the Far East in the amounts required, and it was ordered from the States. The air freight bill on this shipment, I have been told, totaled 1.2 million dollars which was about the cost for 1 day in the Vietnam war at that time.

The island of Taiwan in 1962 experienced a brief epidemic of cholera. The epidemic lasted a total of 6 weeks and the mortality rate was low. However, cholera is a quarantinable disease and ports were closed to products exported from Taiwan. In the 6 weeks of the epidemic the loss of foreign exchange that would normally have been derived from the sale in Hong Kong and Japan of two exportable commodities, bananas and fish, was 6 million dollars, or 1 million dollars/week. This was a loss that was born by the U. S. taxpayer as the USAID support to China at that time required a constant increase in the gross national product.

From the above discourse it is evident that much remains to be learned about cholera. First is the relative importance of the sodium ion transport poison that is present in the cholera stool and likewise the relative importance of "overproduction" of protein-free plasma into the gut lumen. Second is the isolation of the toxic agent(s) with appropriate chemical characterization. Third is the development, if possible, of an antitoxin or toxoid to act against the toxic agent(s). Fourth is the finding of a vaccine that will provide better than 90% protection so that control or eradication is possible. Fifth is improvement of oral therapy so that victims in rural areas do not die before reaching a treatment center.

Summary

Before the study of cholera by U. S. Navy scientists, the mortality rate in untreated cases was 60 to 80% and in treated cases was 20%. The institution of the U. S. Navy method of treating cholera has reduced the mortality rate to nil in the uncomplicated case. Navy scientists have shown that the cholera stool is remarkably constant from patient to patient and throughout the course of disease and averages in milliequivalents per liter, Na^+, 140; K^+, 10; Cl^-, 110; and HCO_3^-, 40. The Navy method of treating cholera is extremely simple and is well suited for use in epidemics in populations who have had no experience with cholera. It relies on measurement of plasma specific gravity by the copper sulfate method. Navy scientists have also shown that there is a sodium transport inhibitor in the stools of the cholera patient and have demonstrated a decreased sodium transport from gut lumen to plasma in the acute phase of cholera. They have also pointed out that, unlike other forms of shock, in cholera the mesenteric circulation must continue since the patient will kill himself by loss of protein-free plasma. SEATO scientists working in East Pakistan have shown that tetracycline and other antibiotics will halve the course of the disease and the fluid requirements for its therapy.

Summario in Interlingua

Ante le tempore del studio de cholera per scientistas del Marina Statounitese, le mortalitate inter non-tractate casos esseva inter 60 e 80 pro cento e in tractate casos 20 pro cento. Le institution del methodo del Marina Statounitese pro tractate cholera ha reducite le mortalitate a zero in non-complicate casos. Scien-

tistas del Marina ha monstrate que le feces in cholera es remarcabilemente constante ab un patiente al altere e a transverso le curso total del morbo. Le valores medie, in mEq per litro es Na^+ 140, K^+ 10, Cl^- 110, e HCO_3^- 40. Le methodo del Marina pro le tractamento de cholera es extrememente simple e ben apte al uso in epidemias afficiente populationes sin experientia con le morbo. Le methodo depende del mesuration del gravitate specific del plasma per le utilisation de sulfato de cupro. Scientistas del Marina ha etiam monstrate que il existe in le feces del patiente con cholera un inhibitor del transporto de natrium, e illes ha constatate un reducite transporto de natrium ab le lumine intestinal al plasma durante le phase acute de cholera. Illes ha signalate, in plus, que—per contrasto con le situation in altere formas de choc—le circulation mesenteric debe continuar in casos de cholera viste que le patiente pote morir in consequentia del perdita de plasma sin contento de proteina. Scientistas de SEATO, laborante in Pakistan Oriental, ha monstrate que tetracyclina e altere antibioticos pote reducer per 50 pro cento tanto le duration del morbo como etiam le requirimentos de liquido in su therapia.

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